GENETIC STUDIES OF ASIATIC CLAMS, *CORBICULA*, IN THAILAND: ALLOZYMES OF 21 NOMINAL SPECIES ARE IDENTICAL

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ABSTRACT

Freshwater clams of the genus *Corbicula*, collected from 40 sites up to 1500 km apart in Thailand, and representing 21 nominal species, show no significant geographic variation at 24 electrophoretically detected allozyme loci and are most probably all referable to the widespread Asian species, *C. fluminea* (Müller, 1774).

Thai *Corbicula* have very little genetic variability: mean number of alleles per locus *(A)* was very low: no variation ($\bar{A}$ = 1.0) was detected in 30% of the samples, in the remainder, $\bar{A} \leq 1.1$; mean percentage of loci polymorphic in each sample, $\bar{P}$ = 4.59% (range: 0.0-12.5%); mean individual heterozygosity, $\bar{H}$ = 0.011 (range: 0.000-0.025) with one outlier where $\bar{H}$ = 0.058). The low level of population variability and very low individual heterozygosity suggest that most of the *Corbicula* in Thailand are facultative self-fertilizers. The small amount of genic diversity detected, and the observed genotype frequencies, are apparently maintained by limited outcrossing, at random with respect to shell phenotype, internal shell color and allozyme genotype.

Eighty-eight percent of the samples, including one referable to *Corbicula fluminea*, cluster at Nei's genetic distance values of $D < 0.01$. Only five samples from northeast Thailand stand slightly apart from the others. These very high genetic similarities, coupled with a lack of significant anatomical variation, provide no support for the recognition of more than one species in our samples. Twenty nominal Thai species are synonymized accordingly with *C. fluminea*; another seven nominal species are candidates for synonymy.

Freshwater clams of the genus *Corbicula* (Bivalvia: Corbiculidae) occur throughout Asia, eastern Europe and Africa. One species, *C. fluminea* (Müller, 1774), is the commonest freshwater bivalve in Asia and has recently been introduced to western Europe, North and South America (Morton, 1986). In the United States it spread rapidly and, as a major economic pest, it has been the subject of considerable study and two international conferences (Britton, 1979, 1986). This research has shown that *C. fluminea* is quite variable in shell size, shape, sculpture and color: the same conchological characteristics upon which most *Corbicula* species have been defined. Morton (1979, 1986) reexamined such conchological variation in the numerous nominal species of *Corbicula* from China and Japan and found that such characters were

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taxonomically unreliable. He found that, when morphological variation was considered together with data on ecology, physiology, demography and reproductive behavior, only two biological species could be recognized in east Asia: freshwater *C. fluminea*, and estuarine *C. fluminalis* (Müller, 1774) (Morton, 1986). He suggested that most of the other nominal Asian taxa are synonyms of these two species. This paper examines this hypothesis as it applies to the nominal species of *Corbicula* in Thailand.

Most of the original species descriptions of Asian *Corbicula* are due to Prime (1864) and Prashad (1930, and references therein). Subsequently, Brandt (1974) cataloged the molluscs of Thailand and recognized 28 species of *Corbicula*, five of them new. These systematists worked almost entirely with conchological characters in defining their species and ignored geographic and ontogenetic variation and other biological attributes. The senior author has recently described variation in *Corbicula* representing 22 nominal species from 40 localities throughout Thailand (Kijviriya, 1990). In agreement with earlier reports, she found considerable conchological variation among clams referable to the different species recognized by Brandt (1974). She also found significant variation in shell size, shape, sculpture and color within and between allegedly conspecific populations. Gross anatomy and dissections of the siphon, digestive and reproductive systems, on the other hand, failed to reveal any taxonomically significant variation between any of the populations sampled (Kijviriya, unpub. data). In this paper, we report on detectable genetic variation within and between these populations and conclude that a major taxonomic revision of Thai *Corbicula* is now required.

Genetic variation in *Corbicula* was studied by electrophoretic surveys of multilocus allozyme patterns. Allozymes have proven extremely useful in recent systematic studies of molluscs. Intrapopulation allozymic variation has been used to establish mating systems (Selander and Kaufman, 1973; McCracken and Selander, 1980; Selander and Whittam, 1983), to reveal the existence of sibling species (Davis, 1983; Staub *et al.*, 1990), to reveal cases of interspecific hybridization (Gould and Woodruff, 1986, 1987, 1990; Woodruff and Gould, 1987) and to study environmental effects on population structure (Koehn and Hilbish, 1987; Nevo, 1988). Studies of interpopulation allozymic variation have revealed the extent of geographic structuring and variation in widespread species (Gould and Woodruff, 1978; Grant and Utter, 1988) showing whether disjunct populations are or are not conspecific (Palmer *et al.*, 1990; Woodruff *et al.*, 1988), revealed the historical pattern of a species dispersal (Mulvey *et al.*, 1988; Woodruff *et al.*, 1986, 1988), and helped define the limits of semispecies in syngameons (Woodruff and Gould, 1980; Woodruff, 1989). The comparison of allozymes among nominal species has supported numerous taxonomic revisions and phylogenetic discussions (Davis *et al.*, 1981; Emberton, 1988; Woodruff and Solem, 1990; Woodruff *et al.*, 1987). Given the demonstrable utility of such data on allozymic variation, this approach was included in our study of the Thai *Corbicula*.

Three research groups have previously reported studies on allozyme variation in *Corbicula*. Smith *et al.* (1979) found no detectable variation at 18 loci in five populations from across North America. In contrast, they found some variation at 12-17 loci in four Asian samples: the proportion of loci that were variable *(P)* in the Philippines was $P = 0.17$-0.23, in Hong Kong, $P = 0.25$, and in Japan, $P = 0.76$. The Asian clams were thus moderately to highly variable and the monomorphism of the introduced North American populations was attributed to genetic drift associated with the founder effect during a single colonization. McLeod and Sailstad (1980) then reported consistent (year-round) low levels of genetic variation at seven loci from one population in eastern U.S.A.: $P = 0.14$, and mean individual heterozygosity, $\bar{H} = 0.005$. They concluded that their genetic observations supported the hypothesis that North American *Corbicula* are self-fertilizing. Subsequently, McLeod (1986) reported a study of variation in other populations from both eastern and western U.S.A. and again found little variation within samples. These earlier reports, especially that showing high genetic variability elsewhere in Asia, suggested that allozymic variation might be used in resolving the relationships among the *Corbicula* of Thailand.

MATERIALS AND METHODS

Clams were collected in 1985-1986 at 40 localities (Fig. 1, Appendix) from various provinces throughout Thailand. Most clams were collected from lotic habitats in the rivers or irrigation canals; exceptions were two lake localities (samples 1 and 23). Some clams were stored in liquid nitrogen immediately and hand-carried to the University of California, San Diego (UCSD) for allozymic screening and protocol development; others were maintained alive at Mahidol University and used for routine allozyme studies. Notes on clam density, shell morphology, and habitat type and water pH were recorded. Distributional and conchological criteria (Brandt, 1974) were used to identify 39 of the 40 samples to the species level. Voucher specimens from each sample were deposited in the Museum at the Center for Applied Malacology and Entomology, Mahidol University, Bangkok. Voucher specimen lot numbers are given in the Appendix.

ELECTROPHORESIS

Proteins from 30 animals from each sample were studied by electrophoretic analysis using horizontal starch gel slabs following the techniques of Selander *et al.* (1971). Prior to electrophoresis, individual clams were thawed and quickly homogenized in 0.1-0.2 ml distilled water. The whole-body homogenate was centrifuged at 4,500 g for 10 min and the supernatant was absorbed onto Whatman No. 1 filter paper wicks (3x9 mm) and inserted into 12% (w/v) horizontal starch gels. Electrostarch Lot 146 (Otto Hiller, Madison, Wisconsin) was used throughout this study. Clams from different samples were run on each gel to facilitate comparisons and sample 17 from the Loei River (northeast Thailand) was used as a control group. Three buffer systems were employed to resolve the 14 enzyme systems and 24 allozymes studied (Table 1).

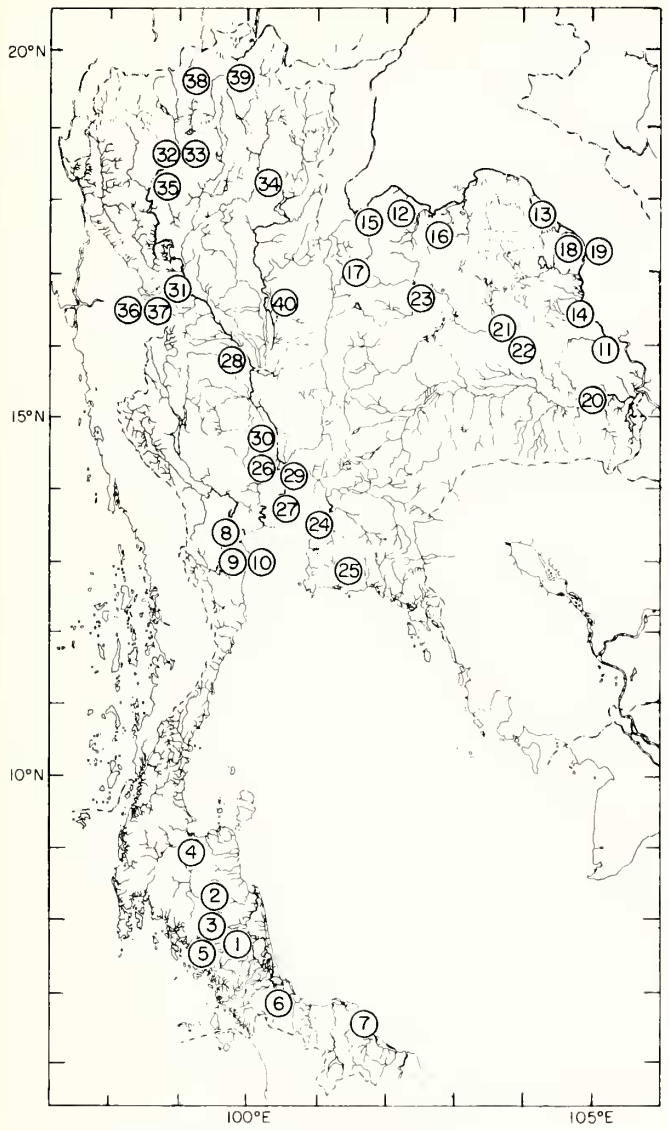

Fig. 1. Location of sample sites in Thailand.

Electrophoresis was carried out at constant voltage for 15 hr by which time a bromophenol blue marker dye had migrated 100-120 mm anodally. Following electrophoresis, each slab was cut into four or five slices and stained for a specific enzyme following standard methods (Murphy *et al.*, 1990). Isozymes were numbered, and allozymes were assigned superscripts (*a*, *b* or c) in order of decreasing anodal mobility. Commonly used enzyme abbreviations are typeset in italics to indicate the presumed genetic locus.

STATISTICAL ANALYSES

Data consisting of multilocus genotypes for individual clams scorable at all 24 loci were analyzed using the BIOSYS-1 computer program (Swofford and Selander, 1981). A locus was considered polymorphic if more than one allele was detected. Mean heterzygosity per individual ($\bar{H}$) was estimated by direct count. Chi-square and Fisher exact tests were used to compare the frequency of individual genotypes with Hardy-Weinberg expectations for a panmictic population. Population structure was examined using fixation indices or *F* - statistics calculated for each locus and sample (Wright, 1978). Nei's (1978) unbiased genetic distance coefficients *(D)* and Rogers' (1972) genetic similarity coefficients *(S)* were calculated and clustered using the unweighted pair group averaging method (UPGMA) for the construction of phenograms and Wagner trees.

RESULTS

We obtained genetically interpretable results for 24 loci per sample. Descriptions of banding patterns and their interpretations are provided by Kijviriya (1990). Allozymic variation in 40 samples from throughout Thailand is summarized in Table 2.

The mean number of alleles per locus ($\bar{A}$) was very low: no variation ($\bar{A} = 1.0$) was detected in 12 of the 40 samples (30%) and in the remaining 28 samples, $\bar{A} \leq 1.1$. The mean percentage of loci that were polymorphic in each

Table 1. Electrophoretic buffers giving optimal resolution of 24 allozymic loci in *Corbicula* sp.

Allozyme (E.C. No.)	Abbreviation	Buffer*
Alkaline phosphatase (3.1.3.1)	*Alp*	TC 6.0
Aspartate aminotransferase (2.6.1.1)	*Aat-1*	TC 6.0
	Aat-2	TC 6.0
Esterase (alpha-naphthyl acetate) (3.1.1.-)	*Es-1*	LiOH
	Es-4	LiOH
	Es-5	LiOH
Glucose-6-phosphate dehydrogenase (1.1.1.49)	*G6pdh*	TC 8.0
Glucose-6-phosphate isomerase (5.3.1.9)	*Gpi*	TC 8.0
Isocitrate dehydrogenase (1.1.1.42)	*Idh-1*	TC 6.0
	Idh-2	TC 6.0
Leucine aminopeptidase (3.4.11.-)	*Lap-1*	LiOH
	Lap-2	LiOH
Malate dehydrogenase (NAD) (1.1.1.37)	*Mdh-1*	LiOH
	Mdh-2	LiOH
Malate dehydrogenase (NADP+) (1.1.1.40)	*Mdhp-1*	LiOH
	Mdhp-2	LiOH
Peptidase (L-leucyl-L-alanine) (3.4.-)	*Pep-A-1*	TC 6.0
	Pep-A-2	TC 6.0
Peptidase (L-leucylglycylglycine) (3.4.-)	*Pep-B-2*	LiOH
	Pep-B-3	LiOH
Phosphoglucomutase (5.4.2.2)	*Pgm-1*	TC 8.0
	Pgm-2	TC 8.0
6-Phosphogluconate dehydrogenase (1.1.1.44)	*Pgdh*	TC 6.0
Xanthine oxidase (1.1.1.204)	*Xdh*	TC 6.0

*TC 6.0: 0.188 M Tris, 0.065 M citrate, adjusted to pH 6.0; diluted 1:9 for gels and 1:5 for electrodes (14 hr, 80 v). LiOH. solution A: 0.03 M LiOH, 0.19 M borate, pH 8.1; solution B: 0.008 M citrate, 0.05 M Tris, pH 8.4; 1A:9B for gels, A for electrode. TC 8.0. electrode: 0.678 M Tris, 0.157 M citrate, adjusted to pH 8.0; gel: 22.89 mM Tris, 5.22 mM citrate, pH 8.0 (14 hr, 100 v).

sample was $\bar{P}$ = 4.59% (range: 0.0-12.5%). Mean individual heterozygosity ($\bar{H}$) was also very low: $\bar{H}$ = 0.011 (range: 0.000-0.025) with one outlier at Mekong River-5 (sample 15) where $\bar{H}$ = 0.058. As 24 loci were surveyed in every sample and as mean sample size per locus was 21-28, our results are adequate to conclude that the Thai *Corbicula* have very little genetic variability.

Minor genetic variation was detected at 28 localities (70%) around Thailand; the remaining 12 were isogenic. Sixteen samples had 1 polymorphic locus, 8 had 2 polymorphic loci, and 4 had 3 polymorphic loci. This variation was detected in 5 loci, four of which were di-allelic and one was tri-allelic. In no case, however, were more than two alleles detected at a locus in a single population. There was no discernible geographic pattern to this variation although levels of *P* were slightly higher in the northeast ($\bar{P}$ = 0.065) than elsewhere (*P* = 0.03 - 0.046).

Where a locus was polymorphic at a particularly locality, the observed genotype frequencies were compared with the frequencies expected under panmixia. Using the chi-square test, the observations were in agreement with Hardy-Weinberg expectations in 40 out of 43 tests ($P > 0.05$). In the remaining three cases, two (both involving *Lap-2*) were also in agreement with Hardy-Weinberg expectation when the Fisher exact test was used. Only one case (involving *Es-5* in sample 15) failed both tests: we detected 6 *AA*, 24 *AB* and no *BB* individuals. This accounts for the elevated heterozygosity noted at this locality.

In the Thai *Corbicula*, we found the simple morphological dichotomy between white and purple internal shell color to be generally inapplicable (Kijviriya, 1990); like Morton (1987) in Hong Kong, we recognized more intermediate color types. However, in eight parts of Thailand we found populations with approximately bimodal distributions of color morphs (samples: 9, 11, 15, 18-19 and 35-37). When these morph-sorted subsamples were compared they were found to be indistinguishable at the 24 allozymic loci. The genes we examined included three of the six loci found to show fixed differences between the contrasting morphotypes studied in Texas by Hillis and Patton (1982).

Table 2. Allele frequencies for variable loci* in 40 samples of Thai *Corbicula*, with summary statistics of genetic variability at 24 loci**.

Sample	*Es-5*[a]	*Lap-2*[a]	*Mdh-1*[a]	*Pep-1*[a]	*Pgdh*[c]	$\bar{N}$	$\bar{A}$	P	$\bar{H}$
1	1.00	0.95	1.00	0.80	0.93	28	1.1	0.13	0.02
2	1.00	1.00	1.00	1.00	1.00	25	1.0	0.00	0.00
3	1.00	1.00	0.83	1.00	1.00	26	1.0	0.04	0.01
6	1.00	0.88	1.00	0.95	1.00	24	1.1	0.08	0.01
7	1.00	0.93	1.00	1.00	1.00	26	1.0	0.04	0.003
9	0.90	0.93	0.92	1.00	1.00	25	1.1	0.13	0.02
10	1.00	1.00	0.88	1.00	1.00	25	1.0	0.04	0.01
11	1.00	1.00	0.97	1.00	0.78	26	1.1	0.08	0.02
12	1.00	1.00	1.00	0.87	1.00	26	1.0	0.04	0.01
13	1.00	1.00	1.00	0.85	1.00	27	1.0	0.04	0.01
14	1.00	1.00	1.00	0.87	1.00	26	1.0	0.04	0.01
15	0.60	0.85	1.00	0.85	1.00	25	1.1	0.13	0.06
16	1.00	0.87	0.93	0.95	1.00	27	1.1	0.13	0.02
18	0.23	1.00	1.00	0.93	1.00	26	1.1	0.08	0.03
19	0.10	0.95	1.00	1.00	1.00	26	1.1	0.08	0.01
20	0.00	0.85	1.00	0.80	1.00	26	1.1	0.08	0.02
21	0.00	1.00	1.00	0.85	1.00	26	1.0	0.04	0.01
22	0.20	1.00	1.00	0.90	1.00	25	1.1	0.08	0.03
24	0.97	1.00	0.85	1.00	1.00	24	1.1	0.08	0.02
25	0.80	1.00	1.00	1.00	1.00	25	1.0	0.04	0.02
26	0.97	1.00	1.00	1.00	1.00	25	1.0	0.04	0.003
28	1.00	0.83	1.00	1.00	1.00	25	1.0	0.04	0.01
31	1.00	1.00	0.78	1.00	1.00	25	1.0	0.04	0.02
32	1.00	1.00	1.00	0.87	1.00	24	1.0	0.04	0.01
33	1.00	0.90	1.00	0.92	1.00	25	1.1	0.08	0.02
34	1.00	0.92	1.00	1.00	1.00	26	1.0	0.04	0.01
35	1.00	0.82	1.00	1.00	1.00	26	1.0	0.04	0.02
36	1.00	0.87	1.00	1.00	1.00	24	1.0	0.04	0.01
39	1.00	0.88	1.00	1.00	1.00	23	1.0	0.04	0.01

*Each variable locus has two alleles except for Pdgh where c/a and c/b occur in samples 1 and 11, respectively. All other loci (see Table 1) were monomorphic.

**Samples described in the Appendix. The following 11 samples are not shown but were identical to sample 2: 4, 5, 8, 17, 23, 27, 29, 30, 37, 38, 40 (i.e. N = 22-27, A = 1.0, P = 0, H = 0), where N = mean sample size per locus, A = mean no. alleles per locus, P = proportion of loci polymorphic, H = mean individual heterozygosity.

We tested the relationship between genic variability *(P)* and local abundance by simple regression analysis. As expected, we found a significant negative correlation between abundance and sample area (r^2 = 0.4). There was, however, no significant relationship between sample area or abundance and *P* (r^2 = 0.13 and 0.09, respectively). Population variability was not simply a function of population size.

We looked for ecological factors that could be associated with locally elevated levels of *P*. The four most variable samples (samples 1, 9, 15 and 16) showed no consistent pattern with respect to water depth, water temperature, pH or habitat type. We are unable to distinguish such sample sites ecologically from sites with isogenic clams.

Genetic relationships between the 40 samples were studied by calculating various measures of genetic distance and clustering these values using various tree building algorithms. A phenogram (not shown) based on Nei's genetic distance *(D)* did not discriminate between almost 90% of the samples; one based on Rogers' genetic similarity resolved the trivial differences between some samples (Fig. 2). Both algorithms show that all the *Corbicula* studied from throughout Thailand are virtually identical to one another at the 24 allozyme loci examined.

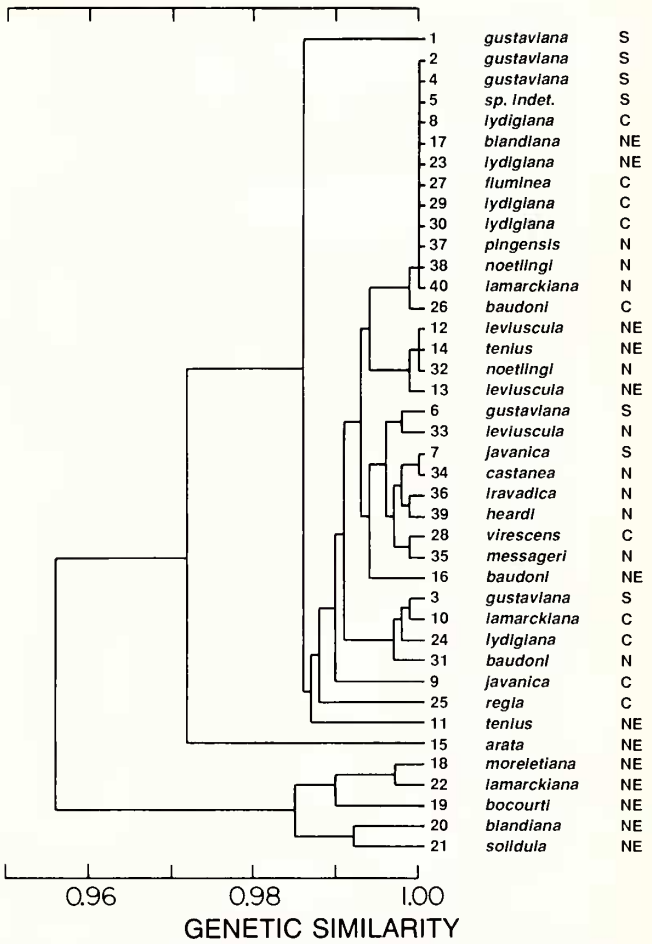

Fig. 2. Dendrogram showing relationships among 40 samples of Thai *Corbicula* generated by UPGMA clustering of Rogers' (1972) genetic similarity *(S)* estimates based on 24 loci. The cophenetic correlation is 0.971.

DISCUSSION

The interpretations concerning allelic variation were made in a very conservative fashion. Genetically interpretable variation was seen in 5 loci: *Es-5, Lap-2, Mdh-1, Pep-A-1* and *Pgdh*. In the majority of cases (39 out of 44), where a locus was polymorphic at a particular locality, only one of the two homozygote genotypes was observed. Both homozygote genotypes were, however, seen in at least one sample for 4 of the 5 polymorphic loci (*Es-5* being the exception). Thus, our genetic interpretations are supported by the observation of all possible genotypes segregating in Thai *Corbicula* in the majority of the variable allozymes.

Another laboratory using different techniques could find variation where we have found none as single-gel electrophoretic surveys are known to fail to detect about 20% of true sample variability (Ayala, 1982; Selander and Whittam, 1983). For this and other reasons, the comparison of electrophoretic results between different laboratories is not recommended (Nei, 1987). However, some comments on the results of Smith *et al.* (1979), the only other published survey of variation in Asian *Corbicula*, are appropriate. Their study involved eight unique loci and ten that were shared with our survey. They too found variation in *Mdh-1* and Pgdh in Asian *Corbicula*. In contrast, where they found *Gpi-1* was variable in samples from Japan, Hong Kong and the Philippines, we detected no variation in Thailand. The other three polymorphic loci that we detected in Thailand were not studied by Smith *et al.* (1979).

The number of individual clams studied per sample and the number of loci investigated per clam are adequate for the estimation of levels of genic variability. The approximate average values of $\bar{A} < 1.1$, $\bar{P} = 0.05$ and $\bar{H} = 0.01$ are all very low relative to those estimated for many other species of invertebrates. For example, Nevo *et al.* (1984) reported that for 361-371 species of invertebrates $\bar{P} = 0.38$ and $\bar{H} = 0.10$. Our estimates for the Thai *Corbicula* are also lower than those obtained by Smith *et al.* (1979) who found P = 0.17-0.76 in the Philippines, Hong Kong and Japan. The average Thai values are thus closer to those estimated for North American populations where $\bar{P} = 0.04$ and $\bar{H} = 0.002$ (data summarized by Britton and Morton, 1986). Such mean values can, however, be misleading: the most variable sample in Thailand (sample 15) had $P = 0.125$ and $\bar{H} = 0.06$ and is thus much more variable than most of the North American populations.

Corbicula fluminea has, at various times, been regarded as dioecious, as monoecious, as a consecutive protandic hermaphrodite, and as a simultaneous hermaphrodite (Kraemer, 1979; Britton and Morton, 1979, 1986). The low level of population variability and very low individual heterozygosity suggest that most of the *Corbicula* in Thailand are facultative self-fertilizers. The reduced variability observed is similar to that found in other self-fertilizing molluscs (Selander and Kaufman, 1973; Selander and Hudson, 1976; Hornbach *et al.*, 1980;

McCracken and Selander, 1980; Stoddard, 1983; Nevo *et al.*, 1984). Morton (1983) recognized that different populations of *C. fluminea* can have different sexual strategies; he noted different degrees of self-fertilizatin in lotic and lentic habitats.

The small amount of genic diversity detected in 70% of the samples must be maintained by either inbreeding among a few strains with limited outcrossing or by the self-fertilization of a large number of heterozygotes. We favor the former explanation as we failed to find the alternate homozygotes in 88% of the situations where we detected heterozygotes (each situation involved a specific polymorphic locus in a specific sample). Occasional random outcrossing would also account for observations that genotype frequencies conformed to Hardy-Weinberg expectations in 98% of the cases and the concomitant lack of a consistent pattern of heterozygote deficiencies expected for obligate self-fertilizing species.

Linkage disequilibrium, which is common in self-fertilizing plants, was not detected in the variable samples of *Corbicula*. There was no significant association between alternate alleles at two or more polymorphic loci in any population. Thus, the variable populations in Thailand are not simply composed of two or more monogenic clones with limited interbreeding. Instead the pattern we discovered suggests amphimixis is occurring today in some populations that have a recent history of automixis. The proportion of progeny produced by self-fertilization *(S)* can be estimated from the proportion of heterozygous individuals in each subpopulation (Hamrick, 1983; Hillis, 1989). Using *Pgdh* genotype frequencies we estimated the frequency of self-fertilization in sample 10 to be 13%; using *Pep-A-1* we estimated the rates to be 29% in sample 1, 29% in sample 20, and 23% in sample 14; using *Lap-1* our estimates were 68% in sample 7 and 57% in sample 16. These results indicate that multiple reproductive modes may be used differentially in different localities.

The very low levels of genic variation and small sample sizes make it difficult to study population structure using Wright's (1978) fixation indices or *F*- statistics. The over-all-loci values of $F_{is} = -0.13$ indicates insignificant inbreeding within each sample. However, when the data are examined on a finer scale, eight significant individual locus F_{is} values were obtained in eight different samples; i.e. $F_{is} > -0.20$ for *Es-5* in four samples, and for *Lap-2* and *Mdh-1*, in two different samples each. In contrast, the larger $F_{st} = 0.46$ indicates significant variation among samples. This value is similar to the average fixation index for self-fertilizing plants ($F_{st} = 0.44$; Hamrick, 1983). The reduction in individual heterozygosity in the Thai *Corbicula* as a whole (due to both substructuring and inbreeding) is also quite substantial ($F_{it} = 0.39$).

The phenetic tree based on Rogers' genetic similarity (Fig. 2) shows how all 40 samples of Thai *Corbicula* are almost identical at the loci studied. In terms of the more widely reported Nei's genetic distances, 88% of the samples (35/40) cluster at averaged genetic distance values of $\bar{D} < 0.01$; such values are smaller than their associated standard erros (Nei *et al.*, 1986). Most of the Thai *Corbicula* are thus genetically identical or indistinguishable at a reasonably large sample of allozyme loci. Only five samples from northeast Thailand stand slightly apart from the others: samples 20-22 from the Mun River drainage and samples 18-19 from the Mekong River at Nakhon Phanom. Their genetic differentiation rests primarily on their having unusually high frequencies of *Est-5^b* and to a lesser extent on their frequencies of *Pep-A-1^b*. Neither of these alleles are unique to these localities or even to northeast Thailand, however, so these samples are differentiated from the others at a barely significant level ($D = 0.03$-0.04). Not only were these regionally commoner alleles found elsewhere in Thailand, but we failed to detect them at two other Mekong River sites (samples 14-15) between Nakhon Phanom and the point 300 km downstream where the Mun River enters the Mekong.

The overall level of genetic differentiation seen among the 40 samples of *Corbicula* is well within that expected for conspecific populations of sexually reproducing species. Thorpe's (1983) survey of 1111 published conspecific comparisons showed that 98% of the genetic distance estimates were $D \leq 0.10$. Self-fertilizing species have received less attention, but generally show more geographic differentiation than outcrossing species, i.e. the observed values for Thai *Corbicula* are much lower than expected for a group of self-fertilizing congeneric species. The very high genetic similarities estimated here provide no support for the recognition of more than one species of *Corbicula* in our samples.

We hasten to add that even within a clade there is no simple relationship between genetic distance and taxonomic level. Some most closely related species are known that are very similar at their allozyme loci (Davis *et al.*, 1981; Gould and Woodruff, 1987). Nevertheless, the preponderance of the evidence on genetic differentiation associated with species of vertebrates (Avise and Aquadro, 1982), invertebrates generally (Nei, 1987), and molluscs in particular (Woodruff *et al.*, 1988), supports the conclusion that our samples are conspecific. Furthermore, the degree of differentiation seen in the Thai *Corbicula* is less than that reported in other species of freshwater bivalve (Davis, 1983; Davis *et al.*, 1981; Kat, 1983; Kat and Davis, 1984). It is significantly less than that reported between clones of other automictic molluscs ($D = 0.06$-0.12) by McCracken and Selander (1980). The lack of genic differentiation in an area the size of Thailand was a little surprising. We failed to detect any evidence for even incipient speciation in clams from different river systems. Had we known, *a priori*, that the Thai *Corbicula* were primarily self-fertilizing we would have predicted even more differentiation between clones; as was found in North American slugs (McCracken and Selander, 1980) and Australian *Thaira* Stoddard, 1983, 1985).

In the absence of comparable allozyme studies of *Corbicula* from elsewhere in Southeast Asia, we are unable to discuss the evolution or phylogenetic relationships of the Thai clams. Their close similarity probably reflects recency of divergence. Time for the accumulation of genetic variation has been limited and self-fertilization has worked constantly to eliminate heterozygotes and rare alleles. The degree of differentiation observed among the Thai *Corbicula* could have evolved in less than 200,000 years if Nei's (1987) moderate calibration of the protein-electrophoretic clock (with a *D* value

of 1.0 equivalent to about 5 million years) is applicable.

The allozymic similarity of the 40 samples of Thai *Corbicula*, together with their lack of taxonomically significant anatomical or conchological variation (Kijviriya, 1990), indicates that all are referable to a single species. Two arguments can be made that this species is *C. fluminea*. First, Morton (1986) and Britton and Morton (1986) have argued that most, if not all, of the Asian freshwater *Corbicula* are synonyms of *C. fluminea*. Second, Brandt (1974) identified *C. fluminea* as occurring at several localities in Thailand and we have studied variation in clams from one of these localities (sample 27) and found that morphologically they are typical of *C. fluminea*. Furthermore, as we have discovered that these clams are genetically indistinguishable from all other *Corbicula* studied in Thailand, we propose the following taxa be synonymized with *C. fluminea* (Müller, 1774):

Corbicula arata (Sowerby, 1877) (Brandt, 1974:313; pl. 27, fig. 73)
C. baudoni Morelet, 1866 (Brandt, 1974:323; pl. 29, fig. 102)
C. blandiana Prime, 1864 (Brandt, 1974:313; pl. 27, fig. 72)
C. bocourti (Morelet, 1865) (Brandt, 1974:314; pl. 27, fig. 80)
C. castanea (Morelet, 1865) (Brandt, 1974:317; pl. 27, fig. 79)
C. gustaviana Martens, 1900 (Brandt, 1974:320; pl. 28, fig. 87)
C. heardi Brandt, 1974:328; pl. 29, fig. 104
C. iravadica Hanley and Theobald, 1876 (Brandt, 1974:323; pl. 28, fig. 91)
C. javanica (Mousson, 1849) (Brandt, 1974:315; pl. 27, fig. 82)
C. lamarckiana Prime, 1864 (Brandt, 1974:316; pl. 27, fig. 76-77)
C. leviuscula Prime, 1864 (Brandt, 1974:326; pl. 28, fig. 95)
C. lydigiana Prime, 1861 (Brandt, 1974:316; pl. 27, fig. 74-75)
C. messageri Bavay and Dautzenberg, 1901 (Brandt, 1974: 327; pl. 29, fig. 100)
C. moreletiana Prime, 1867 (Brandt, 1974:321; pl. 28, fig. 89-90)
C. noetlingi Martens, 1899 (Brandt, 1974:319; pl. 28, fig. 88)
C. pingensis Brandt, 1974:324; pl. 28, fig. 93
C. regia Clessin, 1879 (Brandt, 1974:320; pl. 28, fig. 86)
C. solidula Prime, 1861 (Brandt, 1974:326; pl. 28, fig. 96
C. tenuis Clessin, 1887 (Brandt, 1974:318; pl. 28, fig. 85)
C. virescens Brandt, 1974:324; pl. 29, fig. 101

As Brandt (1974) has provided fully referenced synonyms, notes on conchology, types, localities and distribution, and excellent photographs of representative shells, on the pages and plates cited above, such data are not repeated here. Kijviriya (1990) also provides color photographs of representative shells (see Appendix for specific figure numbers).

These taxonomic conclusions are based on the Thai material examined. We cannot assess the validity of any of these 21 taxa from elsewhere in Asia, e.g. *Corbicula gustaviana* from Sumatra or *C. javanica* from Java. In addition, the following seven nominal Thai species were not collected (*C. cyreniformis* Prime, 1860; *C. gubernatoria* Prime, 1869; *C. occidentiformis* Brandt, 1974; *C. pisidiformis* Prime, 1866; *C. siamensis* Prashad, 1929; *C. vokesi* Brandt, 1974) or were found in inadequate numbers for electrophoretic analysis (*C. erosa* Prime, 1861). We therefore have no opinion as to the validity of these taxa but suspect that they too are junior synonyms of *C. fluminea*.

This, then, is our principal result: the freshwater clams of the genus *Corbicula*, collected from sites up to 1500 km apart in Thailand, show no significant geographic variation at 24 genetic loci and are referable to the widespread Asian species *C. fluminea*. This result would be confirmed if it were shown that the Thai *Corbicula* are closely related to better known *C. fluminea* from Hong Kong and North America. We predict that the Chinese clams will be very similar genetically to those in Thailand ($D < 0.10$). Those of the Philippine islands could show greater differentiation associated with founder effects and perhaps reduced variability as in the case of the introduced North American clams. The reported higher levels of variation in Japan ($P = 0.76$, $\bar{H} = 0.23$; Smith *et al.*, 1979) are paradoxical and require confirmation. Perhaps the radically different level of variation in this peripheral population indicates that the Japanese clams have adopted a different mating strategy to those used elsewhere. Alternatively, the island could have been colonized repeatedly by clams of diverse geographically origin.

In parts of the United States, two morphotypes of *Corbicula fluminea* have been identified based on differences in color of the nacre: white or dark purple. The typical form has colored shells and the dark form has purple shells. Hillis and Patton (1982) found that in Texas the two morphs had fixed differences at six of 26 allozyme loci. McLeod (1986) reported a similar finding. Although Hillis and Patton (1982) and others have used these genetic differences as evidence for the presence of two species in North America, the analyses of Britton and Morton (1986) favor a one-species model. Britton and Morton argued that other conchological, ecological and behavioral traits are associated with this dimorphism in both North America and Hong Kong. These suites of associated characters are apparently local adaptive responses to environmental variables and the contrasting morphotypes are thus better regarded as ecophenotypes. According to Britton and Morton (1986), allozymic variation has now been described in 22 North American populations of the white morphotype; $\bar{P} = 0.04$ (range = 0-19%) and $\bar{H} = 0$ in 17 populations and $\bar{H} = 0.0013$-0.0049 in 5 populations. The 3 samples of the purple morphotype that have been described were all monomorphic ($P = 0$, $\bar{H} = 0$). No other genetic studies have been published, although work in progress on rRNA variation will reopen the question of whether more than one species is present in North America (D. Hillis, pers. comm.).

A paradox to emerge from our study concerns *Corbicula fluminea's* great ecological success despite its innate lack of genetic variability. We have found that one of the commonest bivalves is as genetically depauperate in parts of its native range as it is in areas it has successfully colonized in the last 50 years. Although some workers have maintained that genic variability is positively related to ecological and evolutionary adaptability, it is clear that *C. fluminea's* success is based on a narrow genetic base. McCracken and Selander (1980) found a similar pattern in some European slugs introduced into North America. Homozygous self-fertilizing species of slugs occupied a wider range of habitats and had superior colonizing abilities than some more heterozygous outcross-

ing species. *Corbicula* appears to have such a less variable general purpose genome. Clearly, the larger issues presented by *Corbicula* cannot be resolved from within Thailand; a broader geographic survey of genic variability and breeding systems in this widespread species is now required. Only when this is done will we begin to understand the relationship between genetics, the environment, and *C. fluminea's* marked conchological, ecological and behavioral variation.

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APPENDIX

The 40 samples are listed by number (**1-40**, in boldface type); locality; (relative abundance); and species name according to criteria of Brandt (1974). These sample numbers are placed by locality on the map of Thailand (Fig. 1). Clam abundance was scored of a scale from 1 (rare) to 5 (superabundant), where absolutes densities per square meter are approximately 10, 100, 500, 1000, and >1000. In brackets following each species name, we give the voucher specimen's lot number together with a reference to the color photograph (plate and figure number) of representative shells in Kijviriya (1990). Shells were given Mahidol University, Faculty of Science (MUFS) code numbers and deposited in the Museum of the Center for Applied Malacology and Entomology, Mahidol University.

1. Lake at Lampam, Phattalung province, (5), *Corbicula gustaviana* (MUFS-TH00-155, pl. 2, fig. 8B).
2. Ta Pi River-1, Chawang district, Nakhon Si Thammarat province, (2), *C. gustaviana* (MUFS-TH00-157, pl. 2, fig. 8D).
3. Min canal, Chawang district, Nakhon Si Thammarat province, (2), *C. gustaviana* (MUFS-TH00-156, pl. 2, fig. 8C).
4. Ta Pi River-2, Lampoo island, Muang district, Surat Thani province (3), *C. gustavania* (MUFS-TH00-158, pl. 2, fig. 8E).
5. Tachin River, Muang district, Trang province, (4), *C. sp. indet.* (MUFS-TH00-191, pl. 4, fig. 23).
6. Ngae canal, Khlong Ngae Village, Sadao district, Songkhla province, (4), *C. gustaviana.* (MUFS-TH00-154, pl. 2, fig. 8A).
7. Saiburi River, Saiburi district, Pattani province, (5), *C. javanica* (MUFS-TH00-163, pl. 2, fig. 11A/B).
8. Maeklong River, Muang district, Ratchaburi province, (3), *C. lydigiana* (MUFS-TH00-168, pl. 2, fig. 12C).
9. Phetchaburi River, Muang district, Phetchaburi province, (2), *C. javanica* (MUFS-TH00-164, pl. 2, fig. 11C).
10. Phetchaburi irrigation canal, Muang district, Phetchaburi province, (2), *C. lamarckiana* (MUFS-TH00-172, pl. 3, fig. 13C).
11. Mekong River-1, Khemmarat district, Ubon Ratchathani province, (2), *C. tenius* (MUFS-TH00-189, pl. 4, fig. 21C).
12. Mekong River-2, Huai Klor, Nong Khai province, (3), *C. leviuscula* (MUFS-TH00-176, pl. 3, fig. 14C).
13. Mekong River-3, Phaeng village, Nakhon Phanom province, (3), *C. leviuscula* (MUFS-TH00-177, pl. 3, fig. 14D).
14. Mekong River-4, Muang district, Mukdahan province, (4), *C. tenius* (MUFS-TH00-188, pl. 4, fig. 21B).
15. Mekong River-5, Pakchom district, Nong Khai province, (3), *C. arata* (MUFS-TH00-142, pl. 1, fig. 1).
16. Suei River, near Nong Khai town, Nong Khai province, (5), *C. baudoni* (MUFS-TH00-144, pl. 1, fig. 2B).
17. Loei River, Muang district, Loei province, (1), *C. blandiana* (MUFS-TH00-148, pl. 1, fig. 3A).
18. Pho canal-1, Huai Pho, Nakhon Phanom province, (5), *C. moreletiana* (MUFS-TH00-179, pl. 3, fig. 16A/B).
19. Pho canal-2, Huai Pho, Nakhon Phanom province, (5), *C. bocourti* (MUFS-TH00-150, pl. 1, fig. 4).
20. Mun River, Phibun Mangsahan district, Ubon Ratchathani province, (4), *C. blandiana* (MUFS-TH00-149, pl. 1, fig. 3B).
21. Pan River, Yang Talat district, Kalasin province, (3), *C. solidula* (MUFS-TH00-186, pl. 4, fig. 2).
22. Chi River, Muang district, Mahsarakham province, (3), *C. lamarckiana* (MUFS-TH00-173, pl. 3, fig. 13D).
23. Ubonrat reservoir, Khon Kaen province, (3), *C. lydigiana* (MUFS-TH00-167, pl. 2, fig. 12B).
24. Panthong canal, Panthong village, Chonburi province, (4), *C. lydigiana* (MUFS-TH00-170, pl. 2, fig. 12E).
25. Pra Sae Bon River, Glaeng district, Rayong province, (3), *C. regia* (MUFS-TH00-185, pl. 4, fig. 19A/B).
26. Chao Phraya River-1, Pamoke district, Angthong province, (3), *C. baudoni* (MUFS-TH00-146, pl. 1, fig. 2D).
27. Chao Phraya River-2, Krung Thon bridge, Sanghae area, Bangkok, (3), *C. fluminea* (MUFS-TH00-153, pl. 1, fig. 7A/B).
28. Chao Phraya River-3, Muang district, Nakhon Sawan province, (4), *C. virescens* (MUFS-TH00-190, pl. 4, fig. 22).
29. Chao Phraya River-4, Kwae Noi River, Sena district, Ayutthaya province, (3), *C. lydigiana* (MUFS-TH00-169, pl. 3, fig. 12D).
30. Irrigation canal, Lopburi town, Lopburi province, (3), *C. lydigiana* (MUFS-TH00-166, pl. 2, fig. 12A).
31. Ping River-1, Wuttikul bridge, Tak province, (3), *C. baudoni* (MUFS-TH00-147, pl. 1, fig. 2E).
32. Ping River-2, Naowaraj bridge, Chiang Mai province, (3), *C. noetlingi* (MUFS-TH00-180, pl. 4, fig. 17D).
33. Ping River-3, Naowaraj bridge, Chiang Mai province, (3), *C. leviuscula* (MUFS-TH00-175, pl. 3, fig. 14B).
34. Yom River, Muang district, Prae province, (4), *C. castanea* (MUFS-TH00-151, pl. 1, fig. 5).
35. Kuang River, Muang district, Lamphun province, (3), *C. messageri* (MUFS-TH00-178, pl. 3, fig. 15).
36. Moei River-1, Ban Takham, Tak province, (2), *C. iravadica* (MUFS-TH00-162, pl. 2, fig. 10B).
37. Moei River-2, Mae Sot district, Tak province, (1), *C. pingensis* (MUFS-TH00-183, pl. 4, fig. 18A).
38. Fang River, Fang district, Chiang Mai province, (3), *C. noetlingi* (MUFS-TH00-181, pl. 4, fig. 17C).
39. Mae Lao River, Muang district, Chiang Rai province, (1), *C. heardi* (MUFS-TH00-160, pl. 2, fig. 9B).
40. Kaek River, Wang Thong district, Phitsanuloke province, (2), *C. lamrackiana* (MUFS-TH00-171, pl. 3, fig. 13A/B).